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14. ABSTRACT This project uses a mouse model to examine the effects of shiftwork on the expression of genes that are directly involved in the genesis and progression of breast cancer. Task 1: Shift work simulation experiments in mice have been accomplished. High variability has made it advisable to increase the number of animals from n=5 per group to n=10. Results show that shift work produces a shift in the rhythm of core clock genes and the attenuation of expression of genes involved in cell cycle. A manuscript with these results is currently in preparation. Task 2: Two preliminary experiments have been carried out to assess the grafting success of MCF-7 and MDA-MB-231 tumour cells. We expect to run the full experiment within 8 weeks. Task 4: We have successfully crossed the Clock mutation on to a BALB/c-Foxn1nu background. The pups from this cross were not viable due to the inability of the mothers to lactate their offspring. This, in itself, is a very interesting finding. We have been able to maintain this colony by cross fostering the mutant pups to a lactating heterozygote mother. As a backup we have started to cross the Clock mutation on to a Severe combined immuno-deficient (SCID) mouse background with the expectation that these will not manifest the same problems.					
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INTRODUCTION

The current project uses a mouse model to examine the effect of circadian disruption (shiftwork) on genes that are relevant to the genesis and progression of breast cancer. This project arose in the light of information derived from epidemiological studies suggesting that shiftworkers have a higher incidence of breast cancer [2,3]. This project addresses a very simple question: Does circadian disruption contribute to this increased incidence of cancer in shiftworkers?

In recent times we have gained a good understanding of the molecular mechanisms and components that sustain circadian timing and we now know that each cell contains a circadian molecular oscillator that is tightly synchronized at a tissue specific level. Rhythmicity is synchronized with the external environment through the influence of retinally perceived light on a central oscillator located in the cells of the suprachiasmatic nucleus of the hypothalamus [4].

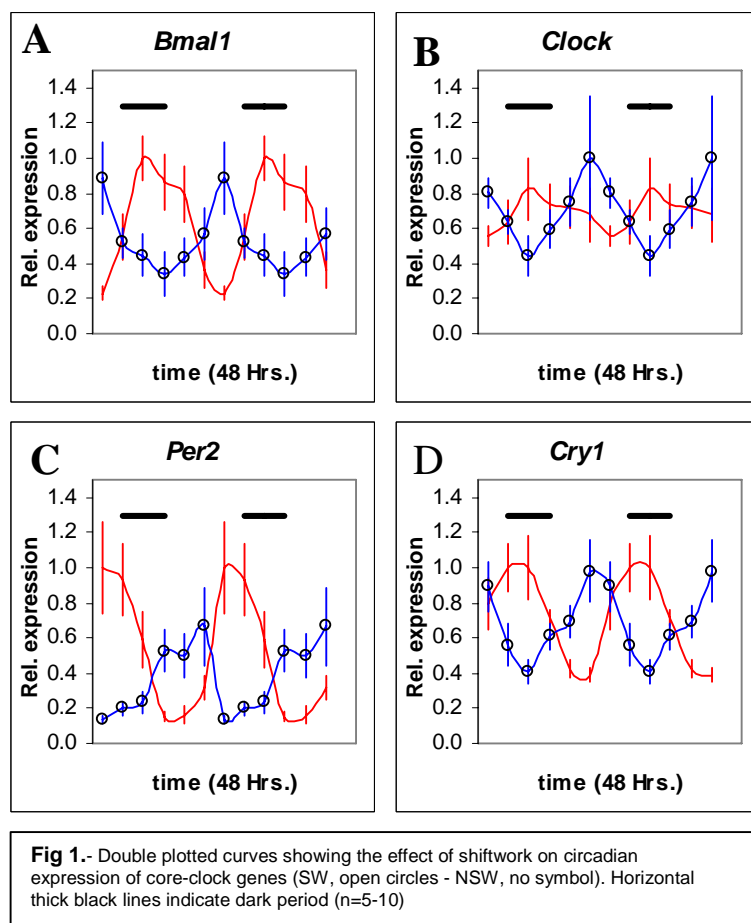
Recent information in the literature examining differential circadian expression of the transcriptome by using high throughput techniques such as microarray suggest that a strikingly high number of genes are directly or indirectly controlled by the cellular circadian clock [1].

Amongst these clock controlled genes are some that are directly involved in the control of cell cycle and apoptosis. Other genes are tumour suppressor genes. Indeed, in a society that is increasingly driven by a 24/7 lifestyle, where a significant proportion of the population is expected to do shiftwork, there is a high probability that critical genes involved in the regulation of cell proliferation and apoptosis are expressed at the wrong time of the circadian cycle or whose rhythmicity is disrupted.

BODY

In accordance with the STATEMENT OF WORK we have undertaken

Task 1.- Expression of clock and clock controlled genes in the mammary tissue of mice under simulated shift work conditions (rapid phase shifting of the LD cycle): To date we have examined the circadian expression of 14 genes under a shiftwork paradigm. In an experiment of shiftwork (SW) vs non-shiftwork (NSW) mice we observed a strong impact of SW on the pattern of gene expression of the clock genes (*Bmal1*, *Clock*, *Per1*, *Per2*, *Cry1* & *Cry2*). These experiments were replicated to improve the power of our statistical evaluation. Our results show that the gene expression pattern of the mice undergoing the shiftworker simulation.



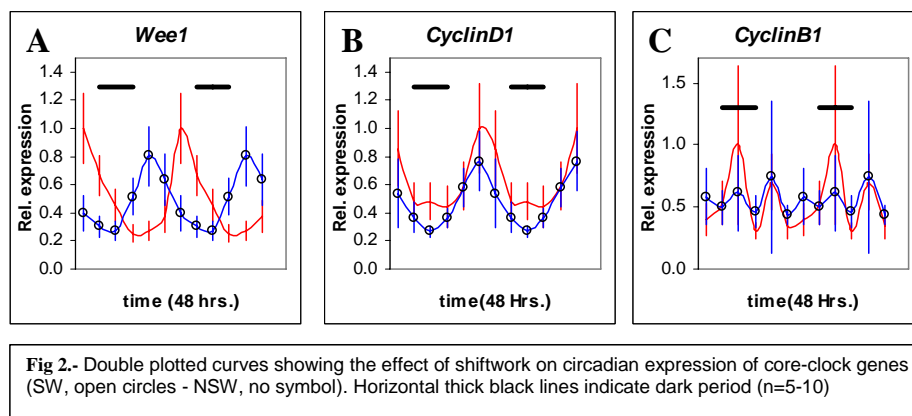
is approximately 180° out-of-phase with respect to the animals on a normal lighting schedule (see fig 1).

With respect to genes that are directly involved in the cell cycle, we have found a rhythm in the expression of *Weel* and *CyclinD1*. Shiftwork has produced a phase shift in *Weel* expression of 6 hours but not in *CyclinD1* mRNA. In contrast, *CyclinB1* did not show a 24 hour variation and there was no significant effect of shiftwork on the pattern of

expression of this gene (see fig 2).

Finally, we have assessed the

pattern of gene expression and the effect of shiftwork on other genes that have been implicated in



the genesis and progression of breast cancer (see fig 3). We did not detect a variation over the 24 hour period for any of these genes.

Consequently, there were no significant

differences in those animals subjected to shiftwork.

We are now in the process of finalizing the analysis of the data and preparing a manuscript for publication of these results.

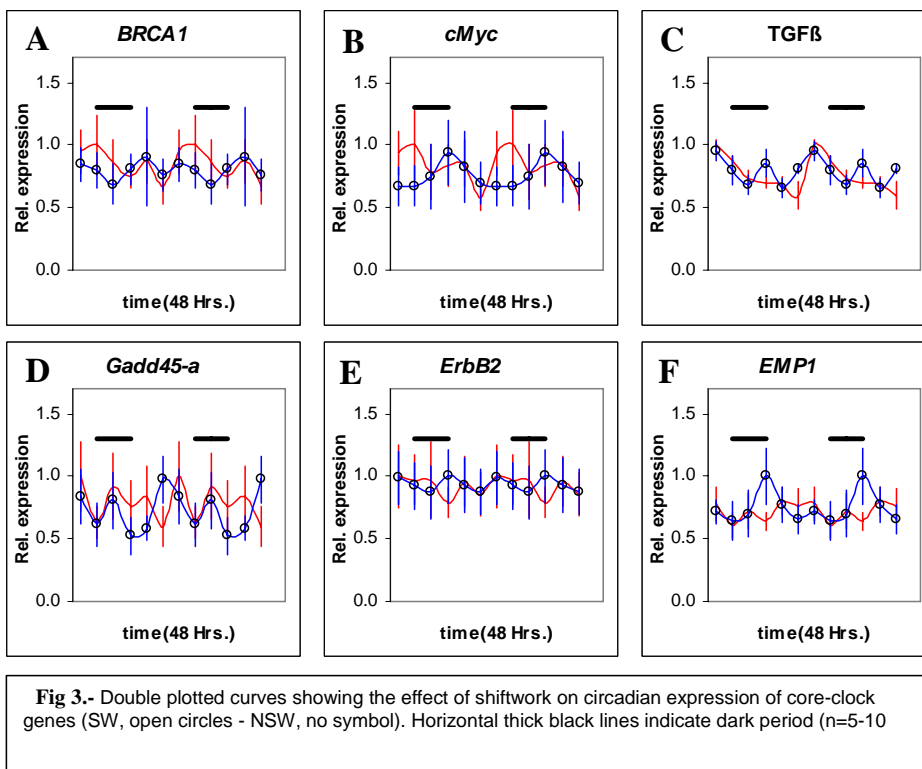


Fig 3.- Double plotted curves showing the effect of shiftwork on circadian expression of core-clock genes (SW, open circles - NSW, no symbol). Horizontal thick black lines indicate dark period (n=5-10)

Task 2 Growth and gene expression of human breast cancer cells in nude BALB/c-Foxn1^{nu} mice under simulated shiftwork conditions: Given the large number of animals involved in each experiment (60 mice) it is imperative that we have a clear idea of the rate of successful grafting of human breast cancer cell lines in the flank of immunosuppressed mice. To date we have run 2 preliminary experiments to assess tumor progression and grafting. In the first experiment 10^5 MCF-7 cells were injected subcutaneously into the flank of 3 animals that were previously implanted with 60-day slow release estradiol pellets (Innovative Research of America[®]). We observed tumor growth in only one animal. In a second preliminary experiment we implanted a higher number of cells (3×10^6) and observed similar results. After consulting with other research laboratories that have successfully carried out similar experiments, we were advised to inject the cells in matrigel[®], an artificial extracellular matrix which has been reported to strongly enhance the grafting capability of these cells. These experiments are currently underway. The latest results are very encouraging, thus suggesting that this portion of the project will be completed within the next 2 months.

Task 4 Development of an immunodeficient *Clock*^{Δ19}+Mel mutant mouse strain. We have successfully generated mice that bear the *Foxn1*^{nu} and *Clock*^{Δ19} mutation. However, the offspring of this cross have proven to be unviable due to the –quite interesting– inability of the mothers to

lactate their offspring. We have been able to expand this colony to a limited extent by cross fostering the pups to heterozygous mothers. This finding is most remarkable and provides very strong evidence that core clock genes play a crucially important role in the development of a functional mammary gland.

These results have lead us to start a new line by crossing the *Clock*^{Δ19} mutation into a severe combined immunodeficient (SCID) mouse background, which is generally believed to be more robust than the NUDE mice. Breeding of this new line is well advanced with the first generation of double mutant mice on the ground and the first experimental animals to be generated by the end of March 2007.

KEY RESEARCH ACCOMPLISHMENTS

Task 1: The assessment of gene expression in the mammary gland under normal and shiftwork conditions. Manuscript in preparation

Task 2: Preliminary experiments for human tumour cell grafting successfully finished.

Task 4: Double heterozygote SCID CLOCK mice to be born by the end of February 2007.

REPORTABLE OUTCOMES

We have found that the expression of *Wee1* is only partially phase shifted in animals subjected to a shiftwork protocol. This contrasts with other genes involved in the cell cycle, such as *CyclinD1*, which in spite of showing circadian variability, do not seem to be linked to the core clock machinery. The relevance of this dissociation of rhythmicity of key genes involved in the cell cycle in the genesis of breast cancer is yet to be determined.

CONCLUSIONS

Task 1 has been accomplished and the data is in the final stages of analysis and the preparation of a manuscript is underway.

Task 2 has been hindered by the inability of the mutant mothers to lactate their offspring. This finding is interesting and has opened a path to new investigations for which we are seeking funding..

Task 4 has advanced slowly due to the difficulty in grafting human cancer cells in NUDE mice. This issue has now been overcome using matrigel[®] and this task will be completed in coming months.

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